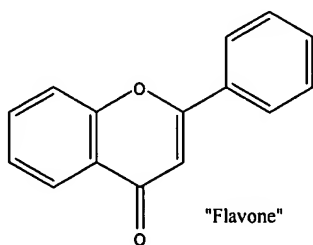## Rejections under 35 U.S.C. § 103(a)

The Examiner has rejected claims 2-8, 10-11, 13-22 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Wenzel as evidenced by Katori, et al. and Hofbauer, et al. (both of which have previously been made of record). Claim 9 has been rejected under § 103(a) as allegedly being unpatentable over Wenzel as evidenced by Katori and Hofbauer in view of Barnes, et al., U.S. Patent No. 5,506,211.

The Examiner's position is that Wenzel discloses all of the limitations of the presently claimed method, except that it does not specifically teach a method for stimulating bone formation and/or inhibiting bone resorption, and that the secondary references fill in this gap. Applicants respectfully traverse this rejection.

According to the Examiner, Wenzel teaches that "compositions of flavone-type compounds of formula I, specifically hesperidin and hesperitin (Table 3) are useful in the treatment of [COX-2] and [NFχB] mediated diseases." As applicants discussed previously, Wenzel relates to the use of flavones for treating COX-2 and NFχB, particularly for treating arthritis and Alzheimer's disease (see ¶0001). Wenzel prescribes the use of flavone compounds for inhibiting the biosynthesis of COX-2 and of NFχB, i.e. as inhibitors of the prostaglandin synthesis. (¶¶ 0001-0005). Numerous compounds that can be used, according to Wenzel, as COX-2 and NFχB inhibitors are shown in Tables 1 to 4 (see pages 3 to 5). According to Wenzel, the diseases linked to COX-2 consist of diseases linked to inflammation, mitogenesis, and ovulation (¶ 0002). Paragraph 0023 specifies a general and theoretical list of diseases which might "potentially" be treated by Wenzel's compounds, including osteoporosis among 16 categories of diseases.

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The secondary references do not cure the deficiencies of Wenzel.   Katori is a

review article on the possible functional roles of COX-2. Notably, Katori indicates that

COX-2 induction or presence has been reported in a wide variety of physiological states,

including "bone absorption." At most, one of skill in the art learns that the presence of

COX-2 is noted in states of bone absorption. This information does nothing to convey to

one skilled in the art the role of COX-2 in bone remodeling, much less whether its

presence, inhibition, or stimulation would have a specific effect on bone remodeling.

Hofbauer describes the role of a receptor activator of NFχB (RANKL) and of NFχB.

Hofbauer is interested in the physiological relationship of RANKL with another receptor

(OPG). With respect to bone diseases, Hofbauer merely discloses that "Abnormalities of

the RANKL/OPG system have been implicated in a wide variety of diseases, including

postmenopausal osteoporosis. Again, it is not made clear whether stimulation or

inhibition would have a particular effect on bone diseases.

The only experimental results disclosed by Wenzel are disclosed in Example 3,

which shows that a specific flavone compound induces an inhibition of the expression

level of the messenger RNA's corresponding to the transcription product of the genes

encoding COX-2 and NFχB. Thus, Wenzel discloses experimental results relating to the

inhibition of the expression of the genes and coding for COX-2 and NFχB by this

flavone:

"Flavone"

(See structure of "flavone" as identified in Table 1 on page 3). The inhibitory effect of this compound on COX-2 and NFχB, the mRNA levels is shown for an *in vitro* final concentration of 150 μM. Wenzel's exemplified compound – "flavone"- does not belong to the group of compounds used in applicants' claimed method. Moreover, as discussed in detail at pages 10-12 of the October 28, 2008 Amendment, Wenzel's "flavone" compound is an aglycone polyphenol, while the compounds of the presently claimed method are glycosylated or sulphated polyphenols, which have very different *in vitro* and *in vivo* behaviors.

Moreover, one of ordinary skill in the art would look skeptically at the results from Wenzel's Example 3 – at least insofar as it might relate to the treatment of osteoporosis – because the gene expression level assay was performed on an HT-29 cell line which consists of a colorectal cancer cell line, which is physiologically of no relevance to bone metabolism.

Furthermore, and very importantly, if a person of ordinary skill in the art were to have followed the Examiner's reasoning that Wenzel suggests using the compounds covered by the present claims to inhibit bone resorption and/or stimulate bone formation, he or she would have only selected those compounds from Wenzel which induce an inhibition of the Cox-2 expression level. That is, Wenzel is clearly directed towards a method of inhibiting Cox-2 (see Wenzel, ¶ [0001]). However, as is shown by the experimental work described in detail in Exhibit A, hesperitin-7-O-gluconoride increases the expression level of the Cox-2 gene by primary cultures of osteoblasts, which are natural cells that are involved in bone metabolism. Thus, contrary to what is suggested by Wenzel, applicants have found that compounds which stimulate Cox-2 actually inhibit

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bone resorption. Nothing in Wenzel or the secondary references teaches or suggests this. Thus, a person of skill in art would not have been led to use the compounds recited in the present claims (hesperidin or a derivative thereof selected from the group consisting of alpha-glucosyl-hesperidin, methyl hesperidin, a conjugate of hesperitin and sulphate and a conjugate of hesperitin and glucuronide) in a method for inhibiting bone resorption and/or stimulating bone formation.
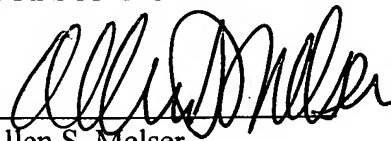
In view of the foregoing, applicants respectfully request that the § 103 rejections over Wenzel be reconsidered and withdrawn.

Conclusion

In view of the foregoing, this application is now in condition for allowance. If the examiner believes that an interview might expedite prosecution, the examiner is invited to contact the undersigned.

Respectfully submitted,

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COX2-ASSAY

A. Materials and Methods

Cell culture

Primary osteoblasts were isolated from calvaria of newborn Wistar rat (INRA, Theix, France) by enzymatic digestion. During all experiments, cells were maintained in α-minimal essential medium (α-MEM) with 10% heat-inactivated foetal bovine serum (FBS) and 1% penicillin/streptomycin. Primary rat osteoblasts were exposure to different conditions.

Hesperetin 7-O-glucuronide (Hp7G) experiments:
- **C-:** minimal medium
- **Hp7G1:** minimal medium supplemented with 1 µM of hesperetin 7-*O*-glucuronide
- **Hp7G10:** minimal medium supplemented with 10 µM of hesperetin 7-*O*-glucuronide

Real-Time PCR

At confluence, cells were exposed for 24 and 48 hours to different mediums: C-, Hp7G1 or Hp7G10. After 24 and 48 hours of treatment, total RNA and proteins were isolated using NucleoSpin RNA/Protein Kit (Macherey-Nagel, Hoerdt, France). Total RNA concentration and purity were measured with NanoDrop spectrophotometer (Wilmington, USA). RNA integrity was checked using RNA 6000 Nano Assay kit with Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, USA). Reverse transcription of RNA was performed using a Ready-To-Go, You-Prime First-Strand Beads Kit (Amersham Biosciences, Piscataway, USA). The SYBR Premix Ex Taq (Perfect Real Time) (TaKaRa, Shiga, Japan) was used to quantify gene expression by Real-Time PCR. The PCR (program: 95°C-30 sec; 40 cycles: 95°C-5 sec; 60°C-35 sec) was performed using a mastercycler ep realplex (Eppendorf, Hamburg,

Germany). Target gene expression was normalized to the housekeeping gene β-actin. The $2^{-\Delta\Delta Ct}$ method was applied to calculate relative gene expression compared to C-condition which corresponds to value 1 (Livak *et al:*, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods. 2001;25:402-8).

Statistics

Non-parametric test Wilcoxon signed rank test (compared to control C-; hypothetical median = 1) on GraphPad® Instat 3 Software (San Diego, CA, USA) was used for the statistical analysis of gene expression.

A p value of less than 0.05 was considered statistically significant.

B. RESULTS

The results of the COX-2 assay are depicted in the appended Figure 1.

The results of Figure 1 clearly show that hesperetin-7-O-glucuronide causes a statistically significant increase ($p < 0.05$) of the Cox-2 mRNA expression level by primary rat osteoblasts, at both concentrations tested (1 μM and 10 μM) after a 48h incubation time period.
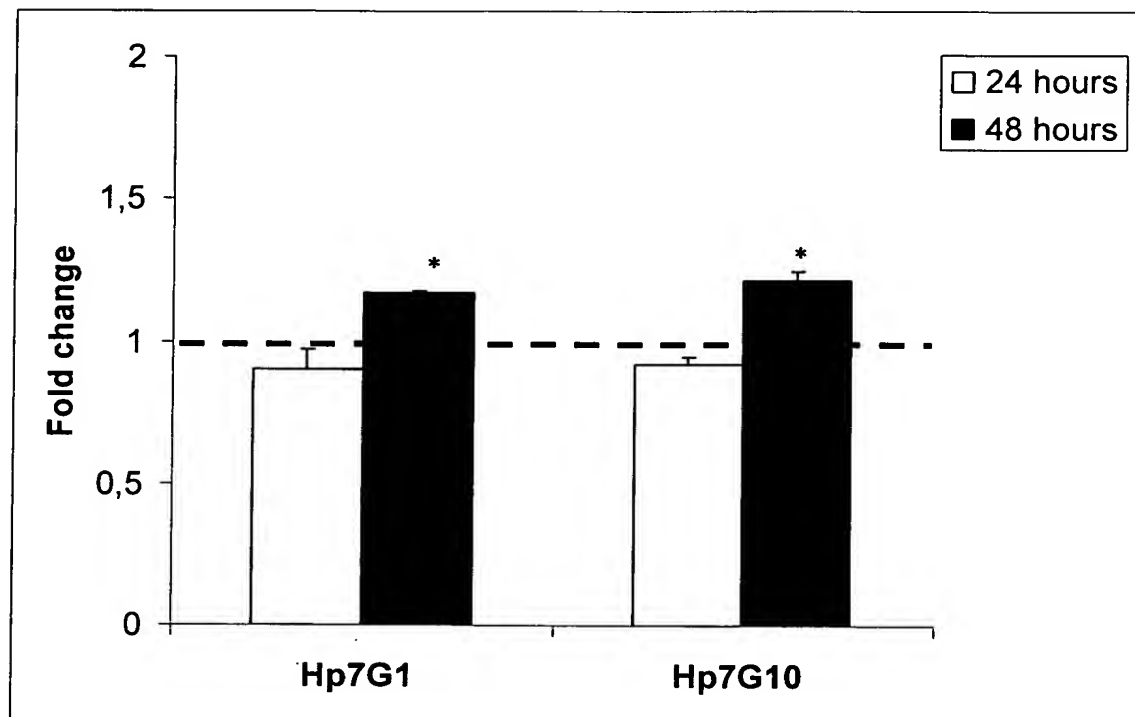
Figure 1